

## Reduced Expression of Cyclooxygenase-2 in Primary Breast Cancer

Recently reported results of a phase 2 study combining the aromatase inactivator exemestane with the cyclooxygenase-2 (COX-2) inhibitor celecoxib for the treatment of breast cancer showed that patients receiving the combined treatment had response rates and clinical benefit rates that were similar to those of the group receiving exemestane only (1). The rationale for using a COX-2 inhibitor for the treatment of breast cancer is based on several reports that COX-2 is frequently overexpressed in breast cancer (2) and that elevated levels of COX-2 in breast cancer specimens were associated with tumor progression and poor prognosis (3). The disappointing effect of celecoxib in this clinical study indicates that COX-2 may not be as crucial for the progression of human breast cancer as previously hypothesized. A possible explanation for the unexpected findings could be that the reported overexpression of COX-2 in human breast cancer was not correct. In one previous study, only the tumor samples were analyzed, and the expression of COX-2 was correlated with clinical parameters such as disease-free survival or lymph node metastasis without comparison to healthy tissue (3). In studies that compared COX-2 expression in tumor and healthy tissues, COX-2 expression was mainly analyzed by immunohistochemistry (4–6). These immunohistochemical methods, however, were not quantitative and would strongly depend on the quality of the antibody and the staining protocol and also on the selection of the analyzed region. Only a few studies analyzed the expression of COX-2 by reverse transcription–polymerase chain reaction (RT–PCR) (4–6), but measurements with conventional RT–PCR are also not quantitative. Recent real-time RT–PCR analyses (7) used just one housekeeping gene for normalization, which could lead to incorrect interpretations because the expression levels of housekeeping genes can vary considerably between tumor tissues and healthy tissues.

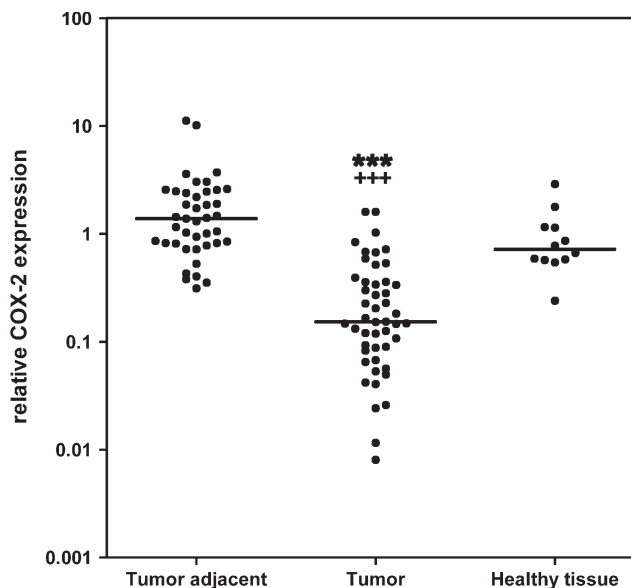
To quantitatively measure the expression of COX-2 in breast cancer tissues,

tumor-adjacent tissues, and healthy breast tissues, we performed real-time RT–PCR analysis and normalized the expression of COX-2 to the mean expression of four different housekeeping genes. The expression of COX-2 mRNA was decreased in the breast cancer samples (mean COX-2 expression: 0.3, 95% confidence interval [CI] = 0.2 to 0.4) in comparison with tumor-adjacent tissues (mean expression: 2.0, 95% CI = 1.3 to 2.7) and healthy tissues (mean expression: 1.0, 95% CI = 0.5 to 1.4) (Figure 1). The median expression of COX-2 in tumor tissues was only 20% of the median expression in healthy tissues ( $P < .001$ ) and only 10% of that of tumor-adjacent tissues ( $P < .001$ ). Furthermore, none of the tumor samples with relative high COX-2 expression exceeded the range of COX-2 expression found in healthy tissue samples or tumor-adjacent samples. Some tumor samples showed only about 1% of the mRNA levels measured in tumor-adjacent tissues.

Our results indicate that COX-2 is expressed at reduced levels in human breast

cancer, not overexpressed as previously reported. Furthermore, we did not find any correlation of COX-2 mRNA expression in the tumor tissues with the mRNA expression of HER2/neu, estrogen receptor alpha, or the progesterone receptor (data not shown). The loss of COX-2 expression in established breast cancers that we observed could explain the failure of celecoxib to inhibit tumor growth in the cited clinical trial (1).

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**Figure 1.** Expression of cyclooxygenase-2 (COX-2) in breast cancer tissues, tumor-adjacent tissues, and healthy breast tissues. Real-time reverse transcription–polymerase chain reaction was performed with custom-designed TaqMan Low Density Arrays (Applied Biosystems, Rotkreuz, Switzerland). Expression profiles of COX-2 and several other cancer-related genes of 48 breast cancer tissues, 41 tumor-adjacent tissues, and 12 breast tissue samples from healthy women obtained during plastic-aesthetic surgery were compared (for detailed description of patients and methods see Supplementary Material, available online). To correct for possible variations in the expression of housekeeping genes, the expression of COX-2 was normalized to the mean expression of four housekeeping genes (*GAPDH*, *UBC*, *TBP*, and *B2M*). Relative expression was calculated with the  $\Delta\Delta C_t$  method. The horizontal line indicates the median relative expression of each group. \*\*\* $P < .001$  vs tumor adjacent tissues, \*\*\* $P < .001$  vs healthy breast tissues.  $P$  values (two-sided) were calculated using the Mann–Whitney test.

## References

1. Dirix LY, Ignacio J, Nag S, et al. Treatment of advanced hormone-sensitive breast cancer in postmenopausal women with exemestane alone or in combination with celecoxib. *J Clin Oncol*. 2008;26(8):1253–1259.
2. Subbaramaiah K, Norton L, Gerald W, et al. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for

- involvement of AP-1 and PEA3. *J Biol Chem.* 2002;277(21):18649–18657.
3. Ristimäki A, Sivula A, Lundin J, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res.* 2002;62(3): 632–635.
  4. Watanabe O, Shimizu T, Imamura H, et al. Expression of cyclooxygenase-2 in malignant and benign breast tumors. *Anticancer Res.* 2003; 23(4):3215–3221.
  5. Half E, Tang XM, Gwyn K, et al. Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma in situ. *Cancer Res.* 2002;62(6):1676–1681.
  6. Yoshimura N, Sano H, Okamoto M, et al. Expression of cyclooxygenase-1 and -2 in human breast cancer. *Surg Today.* 2003;33(11): 805–811.
  7. McCarthy K, Bustin SA, Ogunkolade B, et al. Cyclo-oxygenase-2 (COX-2) mRNA expression and hormone receptor status in breast cancer. *Eur J Surg Oncol.* 2006;32(7):707–709.

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## Notes

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